AN IN-VITRO COMPARATIVE STUDY OF THE PROPERTIES OF DENTAL STONE MODELS DISINFECTED BY INCORPORATION TECHNIQUE AND IMMERSION TECHNIQUE

Dissertation submitted to

THE TAMILNADU Dr. M.G.R.MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH I

PROSTHODONTICS AND CROWN AND BRIDGE

OCTOBER 2017

CERTIFICATE

This is to certify that this dissertation titled "**AN IN-VITRO COMPARATIVE STUDY OF THE PROPERTIES OF DENTAL STONE MODELS DISINFECTED BY INCORPORATION TECHNIQUE AND IMMERSION TECHNIQUE**" is a bonafide record work done by **Dr. PRIYANKAA PRADIP** under my guidance and to my satisfaction during her postgraduate study period of 2014-2017.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY**, in partial fulfilment for the degree of **MASTER OF DENTAL SURGERY** in **Prosthodontics including Crown and Bridge and Implantology**. It has not been submitted (partially or fully) for the award of any other degree or diploma.

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CONTENTS

LIST OF ABBREVIATIONS

ADA - American Dental Association

AIDS - Acquired Immuno Deficiency Syndrome

ANOVA - Analysis of variance

ANSI - American National Standard Institute

ATCC - American Type Culture Collection

BDA - British Dental Association

B_F - Breakage Force

CDC - Centre for Disease Control

CFU - Colony Forming Unit

HBV - Hepatitis B virus

HIV - Human Immuno Deficiency Virus

H_O - Null Hypothesis

HSD - Honesty Significant Difference

Kg - Kilogram

MRSA - Methicillin Resistant Staphylococcus aureus

Mpa - Mega Pascal

SD - Standard Deviation

SLR - Single Lens Reflex

SPSS - Statistical Package for Social Sciences

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"Dental material properties are crucial for the quality of prosthodontic treatment.

Disinfection is critical for a healthy clinical practice".

Every day, each member of the dental team and patients, face potentially harmful, hidden threats from various microorganisms that cover every surface and piece of equipment they come into contact with. The risks from these hazards are frequently overlooked even though they represent a potentially dangerous threat for everyone.

Cross-contamination control[21] is the prevention of transmission of infectious agents between patients and staff within a clinical environment. The dental profession is becoming increasingly aware of the importance of cross-contamination control. To achieve infection control, new products and techniques are constantly being developed. The prosthodontic speciality frequently deals with huge volume of debilitated and immunocompromised patients. Consequently, prosthodontic patients are at a high-risk group relative to their potential to transmit infectious diseases as well as their susceptibility to acquire them. The dental profession must assume that every patient treated is at a high risk of cross infection and should adopt appropriate control measures to break the chain of infection.

Gypsum products are not directly used restorative material in dentistry, but in spite of that they are still considered as a very important adjunctive material that is utilized in a wide range of dental laboratory procedure. According Hishmati RH[15] et al 2002, Gypsum products are widely used materials for the preparation of cast models in dentistry. The cast is a replica of teeth and oral structures. The indirect restoration or an

appliance is fabricated over the cast. So the cast must have reasonable properties in order to withstand the different laboratory steps without being distorted or broken.

Dental casts are transferred several times between the dental laboratory and the dental office. The potential chances of contamination of these models by infectious human pathogens such as Mycobacterium tuberculosis, HIV and HBV has led to the development of more rigorous infection control procedures. It has been proved that bacteria and viruses are transmitted from patients to the gypsum models during the fabrication of the prosthesis, provided if the plaster is poured into contaminated impressions or through contamination of bite blocks and trial bases.

The potential for cross-contamination in prosthodontic practice demands that more attention be given. Measures capable of preventing such transmission of potentially fatal diseases must be in routine use. The use of sterilisation, or surface disinfection, to inactivate infectious agents will reduce the potential for transmission of disease.

The usual solution to this problem has been to rinse the impressions under running water and to place them in an appropriate disinfection solution (ADA[17] Council on Scientific Affairs and Council on Dental Practice, 1996). This should be done upon removal of the impression from the patient's mouth or in the dental laboratory prior to fabrication of cast model. However, two problems may arise. One is the dimensional changes that may arise due to the impressions being soaked in the disinfection. Second is the risk that infectious organisms may still contaminate the gypsum models during the subsequent dental procedures such as jaw registration and the try-in procedures.

There is a wide range of disinfecting methods for the impression materials and gypsum products, which provide a barrier system by controlling infection in dental

laboratory. According to, Adabo GL[2] et al (1999), the commonly followed disinfection techniques are spray technique, immersion technique and incorporation technique. However, immersion of casts has been related by some authors as being deleterious to the final quality of the cast. Spraying the casts with disinfecting solutions has not presented any harmful effects to the surfaces of the plaster casts. However, due to the porosity of plaster, spraying may not disinfect the whole surface of the cast efficiently. Since the disinfection process must be effective without causing alterations on the final quality of the casts, the incorporation of disinfecting solutions in plaster has been regarded as a promising alternative.

The various disinfectants used in prosthodontic laboratory are sodium hypochlorite, iodophors, phenols, glutaraldehyde, chlorhexidine. To ensure the destruction of microorganisms such as hepatitis B and human immunodeficiency virus (HIV), it is best to select a disinfectant solution that is explicitly labelled as having activity against hydrophilic and lipophilic viruses. The most acceptable disinfectant is generally regarded to be 2% buffered glutaraldehyde solution being bactericidal , sporicidal ,fungicidal and virucidal.

Dental casts come into direct contact with impression materials and denture bases that are contaminated by saliva and blood from the patient's mouth. In most incidents, during every prosthodontic appointment cast is left susceptible for cross contamination. The repeated disinfection of impression materials can cause various adverse reactions over the surface of master cast. Therefore, disinfection of dental casts is better than disinfection of impressions, which is effective in preventing cross infection.

This study was carried out to compare the properties of dental stone models disinfected by glutaraldehyde through immersion technique and incorporation technique.

"Let's break the chain of cross-contamination"

AIM

The Purpose of this study is to compare the properties of Dental stone models disinfected by Immersion technique and Incorporation technique. The reduction Microbial contamination and Compressive strength of stone models will be analysed in these two disinfection procedures.

OBJECTIVES OF THE STUDY

The present study was designed with the following objectives:

- To determine the antimicrobial effect of 2% Glutaraldehyde on the Dental stone cast models after two different disinfection methods: Immersion and Incorporation technique.
- To compare the Compressive strength of Dental stone specimens after disinfection by Immersion and Incorporation method.

Following Microbial study and Compressive strength study of Dental stone models, the final objective was to analyse and arrive at the best effective Disinfection method among Immersion and Incorporation technique.

The two null hypotheses assumed were

- Null hypothesis $H^0$1: There is no difference between of two disinfection methods (Immersion method and Incorporation method) on reduction in Microbial contamination on Dental stone cast models.
- Null hypothesis H^0 2: There is no difference in the Compressive strength of Dental stone specimens disinfected by Immersion technique and Incorporation technique.

Norman P Willet[47] (1970) studied that the oral cavity harbours a large and diverse microbial population, with billions of bacteria present in a healthy individual's mouth. The oral cavity is analogous to a continuous culture system. Nutrients are supplied in the form of saliva, gingival fluid, and desquamated epithelial cells. The oral cavity is an initial depository for incoming nutrients and microorganisms. The mouth has ideal conditions for supporting the growth of microorganisms, including aerobic as well as anaerobic bacteria. It is moist, warm (35°-36°) and has an optimum pH (6.8 to 7.2) for most bacterial microbial forms. The variability in the microbial composition in different mouths and between different sites in the same mouth led to early confusion as to which microbes were actually members of the oral flora. The normal oral flora have been considered harmless or having a low order of virulence. However, under suitable conditions, numbers of certain species of the oral flora increase to cause dental caries and periodontal diseases. Furthermore, this flora has the pathogenic potential for causing infections and cross contamination.

Schuster[37] (1973) indicates that the individuals who are at greatest risk from cross infection in dental practice are the health professionals themselves. Because the oral cavity normally contains a diversity of potentially pathogenic microorganisms, the routine use of effective infection control procedures is an important aspect. Antiseptics

and disinfectants are the most widely used of all the drugs in public health practice, in hospital practice and in sanitisation. These agents are used extensively in dental practices and hospitals despite the demonstrably limited effectiveness of certain substances. Various methods of sterilization and disinfection have been suggested. Some of the materials and instruments used in dentistry and the cast cannot be subjected to high heat and hence chemical agents are alternatives to sterilize/or disinfect them. The immersion in a suitable disinfecting solution for an adequate length of time to achieve disinfection is a convenient, inexpensive, and reliable method.

Bonswell P and Olsen I[4] (1974) studied the effectiveness of chlorhexidine in the oral cavity. After rinsing with 10 ml of 0.2% chlorhexidine digluconate for 1 minute was found to be 3.8 mg of Chlorhexidine readily bound to acrylic resin temporary and permanent dentures bases acted as disinfection and reduced spread of infection.

Rowe[32] (1978) mentioned that in the past, the laboratory personnel has been given very little thought that pass from the dental surgery to the dental laboratory. Dental laboratory personnel are now recognizing the importance of efficacious infection control measurements in the handling of contaminated dental materials. Such materials include impressions, casts, occlusal rims, dentures or crown and bridge work that is

taken from the patient's mouth and passed to the dental technician. It is inevitable that these items will be contaminated with the micro-flora of the mouth. Fabrication of stone casts from these impressions or later from contact with occlusal rims that may have been in the patient's mouth may cause cross-contamination between patients and dental laboratory personnel. To disinfect impressions and the stone models have included the use of sodium hypochlorite, glutaraldehyde, iodophor, chlorhexidine, ethylene oxide gas, steam autoclave and ultraviolet rays are being used. The antimicrobial effect on the physical properties of the impressions and the resultant models were the scope of many investigations.

Mitchell A, Robert J[24] (1981) recommended chemical disinfectants such as Cidex, 2% Gluteraldehyde, Idophor solution, and 5.25% sodium hypochlorite diluted to 0.5% to 0.05% with tap water to disinfect dentures, casts, impression trays, mold and shade guides. It was concluded that improved sterile techniques in handling patient's dental models can substantially reduce cross contamination.

Schonfeld[38] (1983) have demonstrated that microorganisms are transferred from contaminated impressions to the surface of the cast and could be measured. American Dental Association (ADA) and the Centers for Disease Control and Prevention have suggested methods for the disinfection of dental casts, including immersion in or

spraying with a disinfectant. It is important that these materials have no effect on dimensional accuracy. Other methods for decontamination of the casts include incorporating chemicals into gypsum at the time of mixing or using die stone containing disinfectant. However, these methods have been reported to affect mechanical properties such as setting time, compressive strength, and dimensional accuracy

The use of soap, soap water, house hold detergents, baking soda, vinegar, ammonia salts, borax, dilute acid, sodium hypochlorite, UV radiation, chlorhexidine, ultrasonic cleansers have been recommended from time to time by the various authorities like **Longwell[22] (1955), Mittleman (1958), Antony and Gibbons (1958), Jorgens (1958), Naylor (1959), Flesh (1960), Peyton and Antony (1965), Smith (1966), Mc McCollum et al (1973), Shannon et al (1976) and Blackstone (1977)**

The properties of ideal disinfectants are as follows.

1. Broad spectrum.

2. Fast acting.

3. Should not get affected by physical factors like organic matters, or soaps or detergents.

4. Surface compatibility

5. Residual effect on treated surface.

6. Easy to use.

7. Odourless.

8. Economical.

Iones[16] ML, Newcombe RS, Barry C et al (1988) studied that many materials are unsuitable for immersion in certain disinfectant solutions, polyether and irreversible hydrocolloids have been particularly well documented, Irreversible hydrocolloid impressions are known to imbibe water when exposed to aqueous solutions. Thus, when an irreversible hydrocolloid material is immersed in a disinfectant solution for a period necessary to destroy pathogens, the dimensional stability is sacrificed and its configuration changed.

Runnells[33] (1988) found that 23 serious infectious diseases, viral and bacterial, have the potential for transmission through the dental practice. Of all these diseases, the Acquired Immune Deficiency Syndrome (AIDS) as well as hepatitis and tuberculosis may have extremely serious complications (Bergman, 1989).Sherwood (1989) ensured the destruction of microorganisms such as hepatitis B and human immunodeficiency virus (HIV), it is best to select a disinfectant solution that is explicitly labelled as having

activity against hydrophilic and lipophilic viruses. The most acceptable disinfectant is generally regarded to be 2% buffered glutaraldehyde solution. Therefore, as glutaraldehyde leads to better decontamination than the other agents, the best solution is to reduce exposure by improving working conditions and practices.

Schutt RW[39] (1989) has outlined a multiple barrier system developed to prevent cross-contamination in the prosthodontic laboratory. The system involves a protocol of specific sequential steps for disinfecting dental prostheses as they enter and leave the laboratory. The primary phase of the system works to attack the infectious organisms, before the impression has been poured, by static immersion in a cold disinfectant solution. The second phase attacks the organisms after the impression has been poured by including disinfectant agents in the liquid with which the powder of the casting medium is to be mixed. Alternatively, a gypsum material containing an antimicrobial agent can be used. The third stage involves exposing the casts to a disinfectant soak. The disinfectant cycle of the initial system, as previously tested, required up to 30 minutes.

Gibbs[12] (1990) showed that the immigration of carriers of M tuberculosis and the susceptibility of AIDS sufferers (a "tag-along" phenomenon), the numbers of patients having active tuberculosis is increasing. Tuberculosis is transmitted by sputum and is

consequently a high risk in a prosthodontic practice. This is especially true when treating older patients, who are particularly vulnerable to infection. To protect against the transmission of M tuberculosis, a routine disinfection with glutaraldehyde solution would be necessary.

Samaranayake[34], Hunjan, Jennings (1990) found that microorganisms can be recovered from impression and cast surfaces even after a 5-hour incubation period. There is a wide belief that impressions may act as a vehicle for microbial transfer from the patient's mouth to dental gypsum models. A visual study of impressions immediately after removal from the mouth often reveals blood clinging to the impression material. Washing the impression sometimes does not clear away all the blood. However, there is no guarantee that all the organisms from the mouth which may possibly be attached to the impression surface have been removed by the washing procedure

Powell GL[31], Runnells RD, Saxon BA, Whisenant BK et al (1990) collected various samples obtained from dentures, impressions, wax occlusal rims and crown and bridge work, and were cultured on their arrival at the dental laboratory to determine the extent of viable organisms present on these items . Results showed that 67% of all materials

sent from dental offices to dental laboratories were contaminated with bacteria of varying degrees of pathogenicity.

Stern[42] et al (1991) stated that, it may be necessary to disinfect the definitive cast at least 7 times (60 min each) with either iodophor or phenol disinfectants from the time of fabrication through insertion of complete or removable partial prosthesis**.** The potential for cross contamination with stone casts is especially present in Prosthodontics because of multiple opportunities for the transfer of infectious agents from blood and saliva to the casts through impressions, record bases, occlusion rims, and trial dentures.

Mansfield[23] and White (1991) stated that the disinfection of plaster models can be carried out through spraying or immersion in a disinfecting solution. The incorporation of antimicrobial agents in the plaster mass and immersion of casts has been related by some authors as being deleterious to the final quality of the cast as spraying them with disinfecting solutions has not presented any harmful effects to the surfaces of the plaster casts. However, due to the porosity of plaster, spraying may not disinfect the whole surface of the cast efficiently**.**

ADA Council[17] (1992) revised its guidelines for incorporation and immersion disinfections. The guidelines recommended are, the chemical agents should be effective

against virus, spores, and bacterial microorganisms. These agents include formaldehyde, chlorine compounds, glutaraldehyde, phenols, and iodophors. Immersion in sodium hypochlorite for 10 min at a concentration of 1:10 dilution (0.525%) is recommended for immersion disinfection.

Owen[29] (1993) stated that the process of disinfection itself should have no adverse impact on the dimensional accuracy and surface texture features of the impression material and resultant gypsum cast. The ideal disinfection procedure must leave the physical and chemical properties of the impression material and gypsum cast unchanged to achieve accuracy of the final prosthesis.

Sofou[41] et al (2002) have shown the presence of bacteria on all impressions, although at a low level. Their study indicated that all the samples cultured from impressions were cloudy after 24 hours of culturing indicating microbial growth. Staphylococcus aureus, Escherichia coli, and Candida albicans were found to survive on alginate and elastomeric impressions. One study showed that 12% of impressions taken from known tuberculosis patients harboured Mycobacterium tuberculosis.

Naimi[28] TS, LeDell KH, Como-Sabetti K, et al (2003) stated that opportunistic pathogens are bacteria that cause a disease in a compromised host that typically would not occur in a healthy (noncompromised) host. Flora normally found in and on the human body, such as Staphylococcus aureus, Escherichia coli, or Candida albicans, can cause an opportunistic infection, as can an organism such as Pseudomonas aeruginosa found in the environment. Methicillin-resistant S aureus (MRSA) is an important nosocomial pathogen that has recently been reported in patients without typical risk factors for nosocomial acquisition (community-associated MRSA). Outbreaks of community-acquired MRSA infection in healthy children and adults have been described worldwide.

Twomey[44] JO, Abdelaziz KM, Combe EC, Anderson DL (2003) suggested the use of chlorhexidine solutions in an aerosol spray in two different concentrations to disinfect dental impressions. The microbiological study showed that impressions treated with a 0.02% chlorhexidine spray showed positive bacterial growth, while those treated with a 0.5% spray showed negative growth after 24 hours and remained clear after 1 week. The problem with spray disinfection is the inability of the solution to completely cover and maintain contact with all of the surfaces of the cast for the required amount of time. Depending on the angle of the spray dispenser, the undercut areas and interproximal surfaces may be missed in the application of the solution.

Abdulla[1] (2006) repeated immersion in tap water or slurry water is strongly discouraged in literature. When soaking or rinsing is necessary, the cast should be rinsed in water saturated with calcium sulfate, not in tap water. On this very basis, we preferred a disinfectant containing calcium as its component rather than water. Abdulla MA also agreed with the notion that, repeated immersion of type III and IV stone specimens in slurry with distilled water and 0.525% sodium hypochlorite, along with drying in air, caused a significant increase in linear dimension and a significant decrease in wet compressive strength. But he stated that, though both solutions caused some degree of damage to surface details for type III and IV stones, the difference was not significant.

Tredwin[43] (2007) investigated the effect of a commonly used immersion disinfectant upon three different impression materials and any subsequent effects on the abrasion resistance, hardness and surface detail reproduction of gypsum casts. The results showed that none of the disinfected alginate specimens could reproduce the 50μm line. Casts produced from the disinfected alginate were significantly less hard than from disinfected addition silicone. Disinfection significantly affected the abrasion resistance of casts. If disinfecting with immersion method, the impression should be made with a

conventional addition-cured silicone if good surface detail reproduction of the impression material and a hard and abrasion resistant type III gypsum cast are required.

Einar Berg, Dodonta[11] (2007) study was to determine if the bactericidal effect of microwaving gypsum casts is maintained at maximum capacity of the oven (16 casts). Batches of 8 and 16 gypsum casts made from in vivo impressions were divided into halves. One half of each cast was microwaved at 900 W for 5 minutes. The remaining halves were left untreated. When assessed for bacteriological growth, the median CFU/mL of the untreated casts was between 105 and 106, while the microwaved casts showed a CFU/mL of 0, indicating that microwaving as described will disinfect gypsum casts even at maximum capacity of the oven.

Sharanbasap[40] (2012) has chosen to evaluate the efficacy of calcium hypochlorite as a disinfecting additive for the gypsum products and its effect on compressive and tensile strength of the set material. It is hypothesized that, the addition of calcium hypochlorite to type V dental stone in sufficient quantity to disinfect the material would have no deleterious effect on compressive or tensile strength. When calcium hypochlorite was added to dental stone, extra mixing water was required to produce a material of nearly same pouring consistency. The samples, which were put to microbiological tests,

showed effective action of disinfectant on Bacillus subtilis. No deleterious effect on compressive or tensile strength could be found after putting the selected samples with calcium hypochlorite.

Satheesh Haralur[36] (2012) stated that alginate impression as well as the dental cast without disinfection harbours lots of bacteria over them. Study emphasizes mere washing of impression in water is not an efficient disinfection method. Hence, it is imperative on the part of the clinicians to disinfect the alginate impression before sent to laboratory. Bacterial colonies on the corresponding dental cast are dependent on impression disinfection procedure; some dental cast showed increase in number of bacterial colonies compared to source disinfected impressions. Hence additional disinfection procedure for the dental cast can be justified to completely eliminate the cross infection.

Daher Antonio[9] et al (2013) compared the dimensional stability of casts obtained from addition silicone and polyether impressions that were immersed for 10 minutes in a solution of 0.2% peracetic acid or 1% sodium hypochlorite. There was not a significant statistical difference between addition silicone and polyether impressions regardless of the disinfectant materials. It can be concluded that disinfection with the proposed agents

did not produce significant alterations of the impressions and the peracetic acid could be considered a reliable material to disinfect dental molds.

Gloria, Fernada[13] (2014) study aimed to evaluate whether chlorhexidine mixed with irreversible hydrocolloid powder decreases microbial contamination during impression making without affecting the resulting casts. Surface roughness and dimensional stability of the casts were evaluated. They concluded that Chlorhexidine with water substitute during impression taking offers decreased microbial contamination with no negative alterations of the resulting casts, thus providing an easy method for controlling cross-infection.

This Thesis evaluates the effectiveness of disinfection of dental stone models by Immersion method and Incorporation method. The materials and methods used in this investigation are sequentially described in this section.

GROUP I- EVALUATION OF REDUCTION IN MICROBIAL CONTAMINATION

MATERIALS

S NO	PROCEDURE	MATERIALS	BRAND
1	MAKING OF IMPRESSSIONS	Irreversible hydrocolloid impression material- Alginate	Tropicalgin,Zhermack,Italy
2	INTENTIONAL CONTAMINATION OF IMPRESSIONS	MICRO-ORGANISMS	P.aerugenosa S.aureus C.albicans
3	POURING OF CAST MODELS	Type III Gypsum- Dental stone	Kalstone,Kalabhai,Vikroli, Mumbai,India
4	DISINFECTION OF CAST MODELS	2% Glutaraldehyde	Merck Glutaraldehyde solution, Merck specialities private Ltd, Mumbai, India.

5	MICROBIAL STUDY	CULTURE MEDIA	Mc.conkey Agar Human Blood Agar ,Becton Dickinson and Company, USA

ARMAMENTARIUM

SNO	PROCEDURE	INSTRUMENT	BRAND
1	MAKING OF IMPRESSION	Stainless steel perforated dentulous trays	Dentsply, USA
2		Bowl,spatula, scoop, measuring jar	Zhermack,Germany
3		Typodont	Nissin 200-M Typodont jaw, Kyoto, Japan
4		Weighing machine	Essae – Teraoka Koramangala ,Bengaluru Karnataka - India
5		Glass jar	Micro-media,Australia

6	POURING OF CAST MODELS	Straight plaster spatula	API, Germany
7		Bowl	Classic and Unident,India
8		Vibrator	Unident,New Delhi,India
9		Base former	Gresco products, Stafford, USA
10	MICROBIAL STUDY	Culture plates	Micro-media, Australia
11		Inoculation loop	Hi-media labs , Mumbai, India
12		Laminar flow	Tecnico laboratory, Mumbai, India
13		Sterile swab	Hi-media,Mumbai,India
14		Incubator	Remi,Goregon,India

15		Microscope	Labmomed , Los angles USA

GROUP II EVALUATION OF COMPRESSIVE STRENGTH

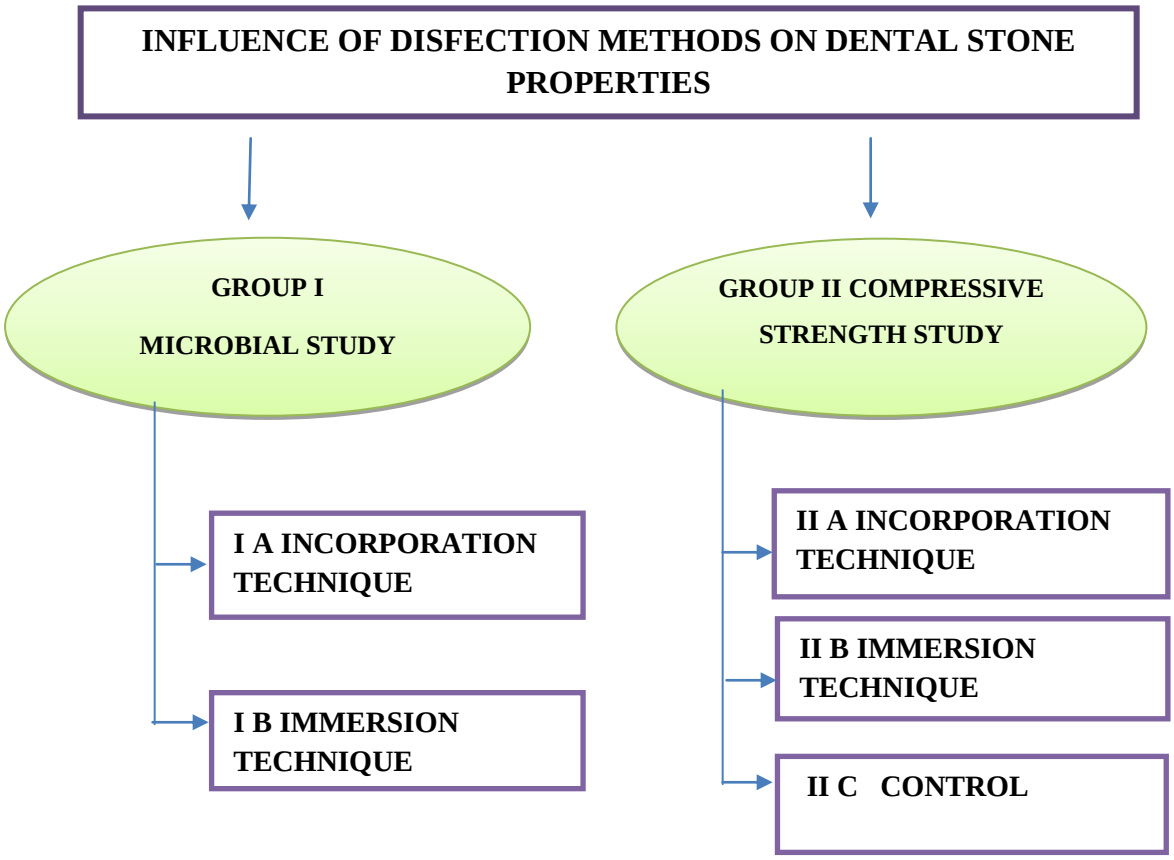
MATERIALS

S.NO	PROCEDURE	MATERIALS	BRAND/MANUFACTURER
1	PREPARATION OF DENTAL STONE SPECIMENS	Type III Gypsum-Dental stone	Kalstone, Kalabhai,Vikroli, Mumbai, India.
2	DISINFECTION OF DENTAL STONE SPECIMENS	2% Glutaraldehyde	Merck Glutaraldehyde solution, Merck specialities private Ltd, Mumbai, India.

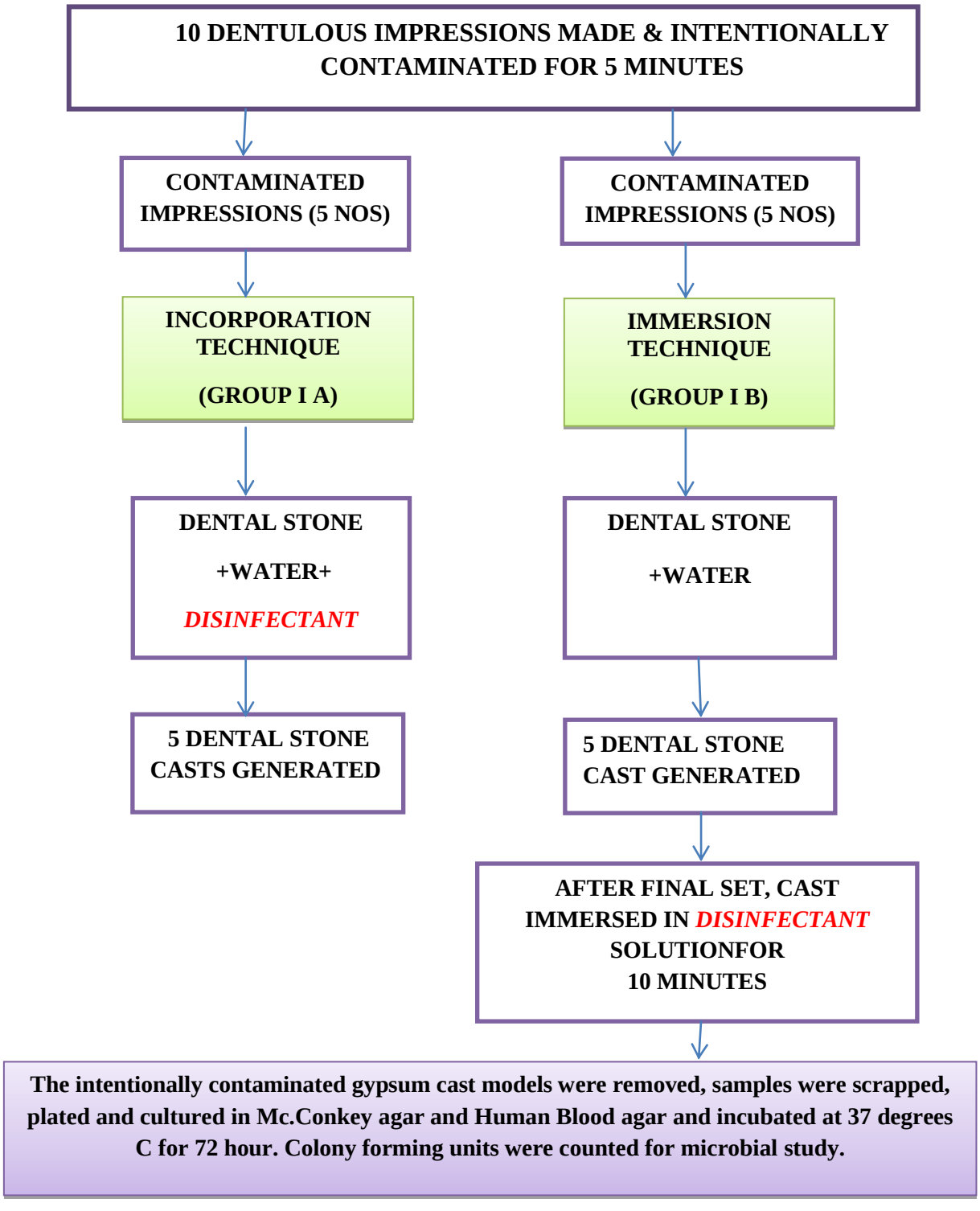
ARMAMENTARIUM

S.NO	PROCEDURE	INSTRUMENT	BRAND
1	PREPARATION OF DENTAL STONE SPECIMENS	Straight plaster spatula	API, Germany
2		Bowl	Classic and Unident New Delhi ,India
3		Vibrator	Unident,NewDelhi, India
4		Stainless steel metal two-piece die	PSG Tech, Coimbatore, India
5	TESTING THE DENTAL STONE TEST SPECIMENS	Universal testing machine	Instron 8801, hydraulic press, Chennai, India

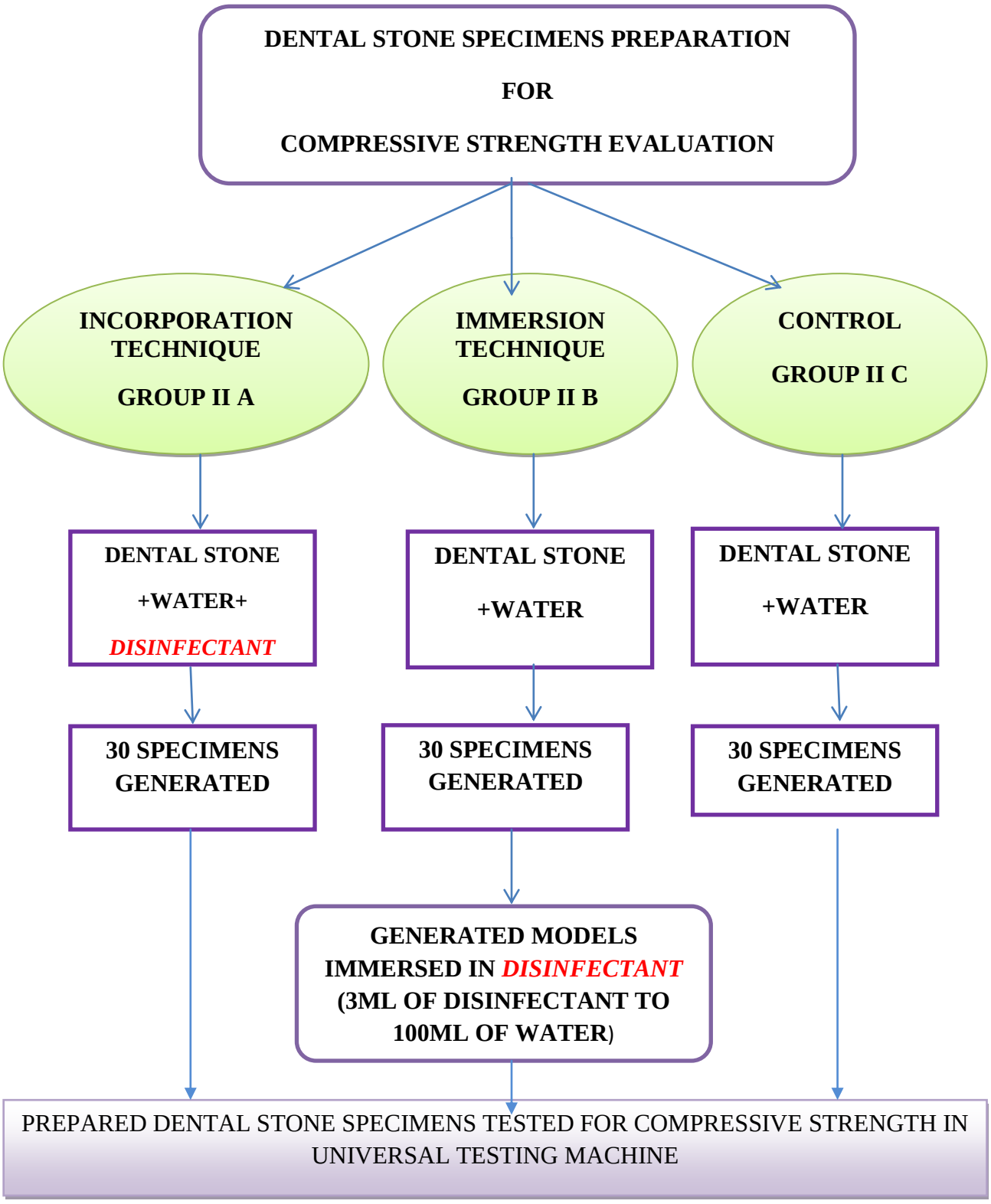
STUDY DESIGN AND SAMPLING



GROUP I- EVALUATION OF REDUCTION IN MICROBIAL CONTAMINATION



GROUP II - EVALUATION OF COMPRESSIVE STRENGTH



METHODOLOGY:

GROUP I- EVALUATION OF REDUCTION IN MICROBIAL CONTAMINATION

1. Making of the primary impression with Alginate followed by intentional contamination
2. Preparation of cast models with Dental stone.
3. Disinfection of Dental stone cast models with 2% Glutaraldehyde by two methods
    - GROUP I A- Incorporation technique
    - GROUP I B- Immersion technique
4. Microbial study of both GROUP I A and GROUP I B disinfected Dental stone cast models

GROUP II - EVALUATION OF COMPRESSIVE STRENGTH

5. Preparation of Dental stone specimens.
6. Disinfection of Dental stone specimens with 2% Glutaraldehyde by two methods.
    - GROUP II A- Incorporation technique
    - GROUP II B- Immersion technique
    - GROUP II C- Control group
7. Testing the Dental stone specimen for dry Compressive strength.
8. Statistical analysis and comparison of two disinfection techniques
9. Results

GROUP I- EVALUATION OF REDUCTION IN MICROBIAL CONTAMINATION

MAKING OF IMPRESSION:

A Nissin 200-m typodont upper jaw (Fig.9) was used in the fabrication of definitive casts. The impression was made with irreversible impression material Alginate[30] (Tropicalgin, Zhermack). Two scoops of Alginate (Fig.1) were added to specified quantity of water according to standard[19] water–powder ratio (16g-38ml). Then it was mixed well with curved spatula that is sufficiently flexible to adapt well to the wall of the mixing bowl with vigorous 8-motion for 45s-1minute. Then the Alginate mixture was placed in the perforated stainless steel dentulous impression tray (Dentsply) and impression was made over the typodont jaw. The impression was then removed from the typodont jaw after 3 minutes of gelation process. (Fig.10, 11)

INTENTIONAL CONTAMINATION:

Three standard strains were used for intentional contamination. Standard strains of Pseudomonas aeruginosa (ATCC 9027) as persistent species[7], Staphylococcus aureus (ATCC 6538) as a vegetative bacterial strain, as well as Candida albicans (ATCC 10231) as a fungal strain were used in this study. The impressions were rinsed with sterile distilled water and then inoculation with 0.1 ml of 18 hour incubated broth of Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans (Fig12, 13). Then impression was poured with dental stone.

POURING OF IMPRESSIONS

- ***GROUP I A STONE CAST MODELS – INCORPORATION METHOD***

In group IA stone casts, 2% Glutaraldehyde aqueous disinfectant solution[12] (Fig.4) was used along with water during mixing of dental stone (Kalstone, Kalabhai Fig.2). The standard water powder ratio for type III Gypsum dental stone is 0.28-0.30(i.e. 28-30ml of water per 100mg of dental stone).In this Incorporation disinfection method, standard water powder ratio of dental stone was modified by replacing 10% of liquid ratio with disinfectant aqueous solution of 2% Glutraldehyde solution(i.e. 3ml of disinfectant + 27ml of water + 100gms of dental stone powder).Incorporating 10% disinfection solution into the gypsum mixture has been reported to be effective without compromising their properties. This amount was doubled considering the size of the typodont and stone casts. Then the impressions were poured. The casts were separated from impressions after 1 hour and allowed to dry for 24 hours (Fig.14).

- ***GROUP I B STONE CAST MODELS –IMMERSION METHOD***

IN GROUP I B casts were poured in intentionally contaminated impressions with dental stone mixed according to ADA specification no.25 with standard[18] water powder ratio 28-30ml water per 100gm of dental stone (Fig.15).The casts were removed after 1hour. Then stone casts were disinfected by immersing in 2% Glutaraldehyde solution for 10-13 minutes (Fig16). The disinfected stone casts were allowed to dry for 24 hours.

MICROBIAL STUDY

The set cast surfaces were swabbed (Fig 3, 17, 18) and scraped under laminar flow chamber environment after 24 hours. The samples were placed in sterile culture plates [3] (micro-media) with Mc.Conkey agar and Human blood agar (Fig 7, 19). It was then incubated (Remi incubator) aerobically at 37 degrees for 72 hours (Fig 7, 19). Then the culture plates were examined visually for growth of colonies and subsequently colony forming units were counted. The Microbial growth (Fig 20, 21) was counted in colony counter under microscope (Fig.5), against comparison with Mc.Farland standard of turbidity of microbial contaminant.

GROUP –II EVALUATION OF COMPRESSIVE STRENGTH

PREPARATION OF DENTAL STONE SPECIMENS

The Dental stone specimens[10] were prepared according to the ADA standard specification no.25 for dental gypsum products. A 40mm high two-piece rectangular stainless steel die (Fig 22, 23, 24) with a cylindrical hollow mold cavity of 20±2mm internal diameter (Fig 25, 26, 27) was fabricated. Accurate orientation of metal die was possible because of key and key way mechanism. The dimension of metal die was in accordance with ADA standard dimensions for compressive testing. The metal mould was placed over glass slab and then dental stone mixture was poured into the cylindrical mould space under gentle vibration.

- ***GROUP II-A -INCORPORATION DIE SPECIMENS***

  In this group, 2% Glutaraldehyde was incorporated during mixing of dental stone for fabrication of dental stone die specimens (Fig 28). The standard water powder ratio for type III Gypsum Dental stone is 0.28-0.30(i.e. 28-30ml of water per 100mg of dental stone). In this Incorporation disinfection method, standard water powder ratio of dental stone was modified by replacing 10% of liquid ratio with disinfectant aqueous solution of 2% Glutaraldehyde solution (i.e. 3ml of disinfectant + 27ml of water + 100gms of dental stone powder). The stone mix was poured in to the metal die mould.

- ***GROUP II-B -IMMERSION STONE SPECIMENS***

  In this group, dental stone specimens (Fig 30) were prepared with standard water powder ratio and mixing was done according to ADA specification no 25.One hour after the die specimen preparation, the stone specimens were immersed[5] (Fig 31)in 2% Glutaraldehyde for 10-13 minutes and then dried for 24 hours.

- ***GROUP II-C -CONTROL STONE SPECIMENS***

  Dental stone specimens (Fig 29) were prepared with standard water powder ratio and mixing was done according to ADA specification no 25.

TESTING THE DENTAL STONE SPECIMENS

The dry compressive strength[35] for the three groups of stone specimens after 24 hours was tested in universal testing machine (Fig 32, 33) at a crosshead speed of 1mm per minute standardisation. The samples of the three groups were transferred to the universal testing machine and specimens were stressed with increasing loads until it fractured. The value obtained at fracture of each specimen was recorded.

MATERIALS AND ARMAMENTARIUM

FIG.1.MATERIALS USED FOR IMPRESSION MAKING

FIG.2.MATERIALS USED FOR PREPARATION OF GYPSUM STONE SPECIMENS

FIG.3.MATERIALS USED FOR MICROBIAL STUDY

FIG.4.

2% GLUTARLDEHYDE

DISINFECTANT

FIG.5.MICROSCOPE

FIG.6.WEIGHING MACHINE

FIG.7.INCUBATOR

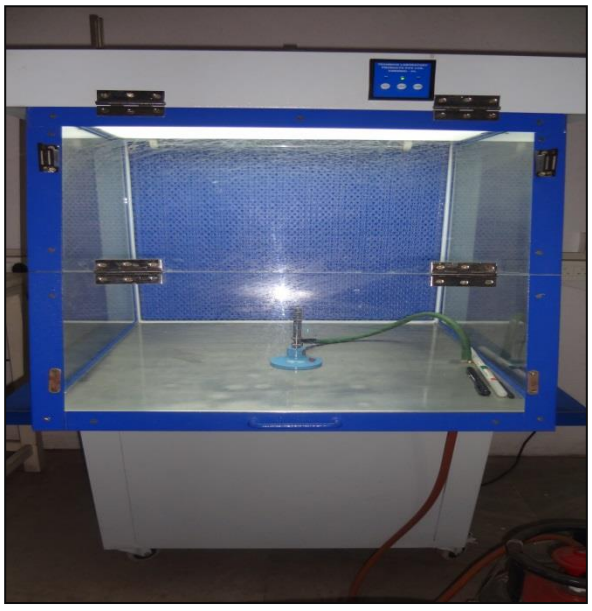


FIG.8.LAMINAR FLOW

MICROBIAL STUDY

FIG.9.TYPHODONT NISSIN 200M

FIG.10.ALGINATE IMPRESSIONS MADE FOR GROUP I A

FIG.11.ALGINATE IMPRESSIONS MADE FOR GROUP I B

FIG.12.INTENTIONAL CONTAMINATION OF GROUP I A ALGINATE IMPRESSIONS

FIG.13.INTENTIONAL CONTAMINATION OF GROUP I B ALGINATE IMPRESSIONS

FIG.14.CONTAMINATED GYPSUM CAST MODELS GROUP I A POURED WITH WATER+DISINFECTANT+ DENTAL STONE

FIG.15.CONTAMINATED GYPSUM CAST MODELS GROUP I B WHICH ARE TO BE DISINFECTED SUBSEQUENTLY BY IMMERSION TECHNIQUE.

FIG.16.IMMERSION DISINFECTION OF CAST MODELS.

FIG.17.MICROBIAL SAMPLE COLLECTED FROM CAST MODEL.

FIG.18.CULTURE PLATES IN LAMINAR FLOW CHAMBER

FIG.19.CULTURES PLATES IN INCUBATOR FOR 72 HOURS.

FIG.20.MICROBIAL GROWTH IN GROUP I A CULTURE PLATES

FIG.21.MICROBIAL GROWTH IN GROUP I B CULTURE PLATES

COMPRESSIVE STRENGTH STUDY:

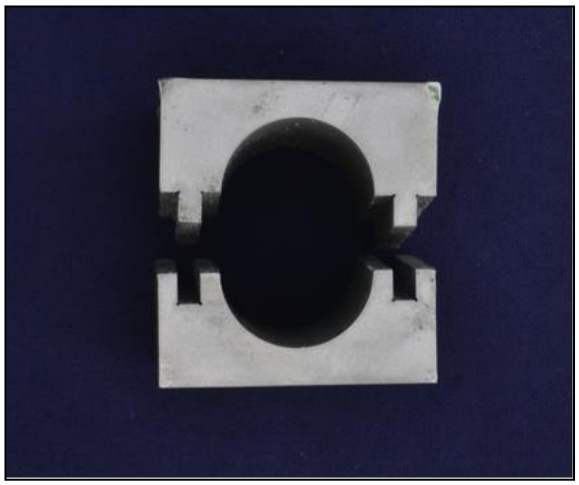


FIG.22.TWO - PIECE STAINLESS STEEL METAL MOLD WITH KEY AND KEYWAY MECHANISM.

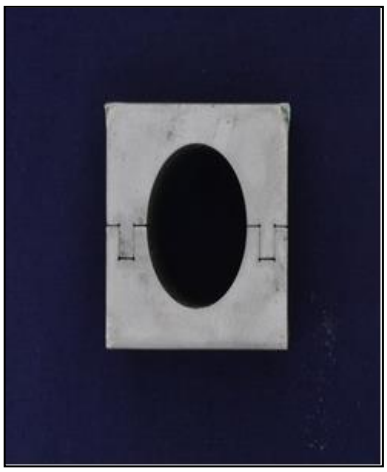


FIG.23.SPLIT METAL MOLD – TOP VIEW

FIG.24.SPLIT METAL MOLD – SIDE VIEW.

DENTAL STONE SPECIMEN

FIG.25.DENTAL STONE CYLINDRICAL SPECIMEN.

FIG.26.DIAMETER OF STONE SPECIMENS – 20mm

FIG.27.LENGTH OF STONE SPECIMEN – 40mm

DENTAL STONE CYLINDRICAL SPECIMENS (DRY) AFTER 24 HOURS :

FIG.28.GROUP II A – DENTAL STONE TEST SPECIMENS AFTER DISINFECTION BY INCORPORATION TECHNIOUE.

FIG.29.GROUP II C – DENTAL STONE CONTROLLED SPECIMENS

FIG.30.GROUP II B IMMERSION OF TEST SPECIMENS IN DISINFECTANT – 2% GLUTARALDEHYDE

FIG.31.GROUP II B DENTAL STONE TEST SPECIMENS AFTER DISINFECTION BY IMMERSION TECHNIQUE.

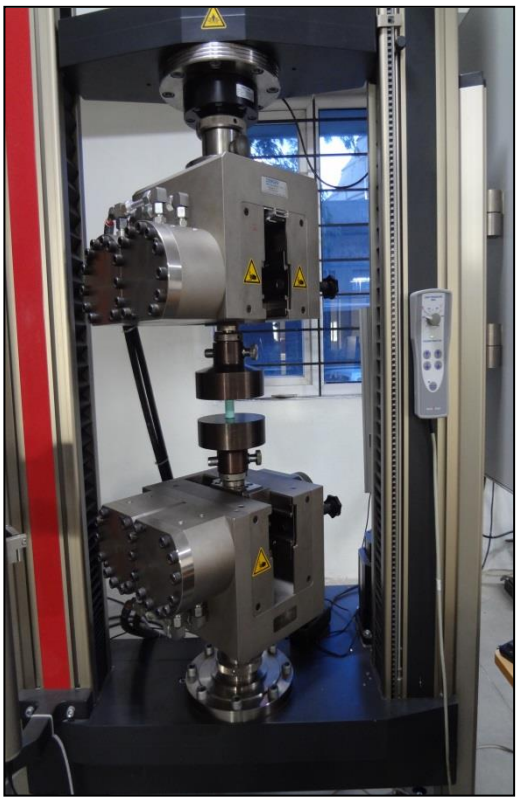


FIG.32.TEST SPECIMEN MOUNTED IN UNIVERSAL TESTING MACHINE.

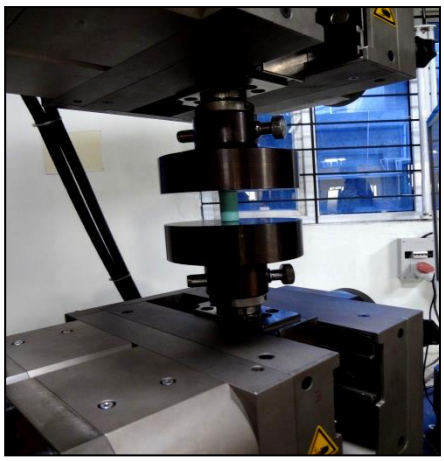


FIG.33.TEST SPECIMEN CRUSHED IN UNIVERSAL TESTING SPECIMEN.

The study was conducted

- To determine the antimicrobial effect of 2% Glutaraldehyde on contaminated dental stone models by two different disinfection methods: Immersion and Incorporation technique.

- To compare the compressive strength of type III dental stone cast models after disinfection by Immersion and Incorporation method.

IN GROUP I MICROBIAL STUDY

The set cast surfaces were swabbed, scraped after 24 hours, and cultured. Then the culture plates were examined visually for growth of colonies and subsequently colony forming units were counted. The microbial growth was counted using colony counter, against comparison with Mc.Farland standard of turbidity of microbial contaminant

TABLE 3: Number of microbial colonies after disinfection by Incorporation technique in Group I A

Number of colonies seen after 72 hours at 10^{-4} dilution

S NO.	CFU/ml
1	0
2	0
3	4
4	9
5	7

The mean colony forming units counted after incorporation of disinfectant in cast model is 4 CFU/ml

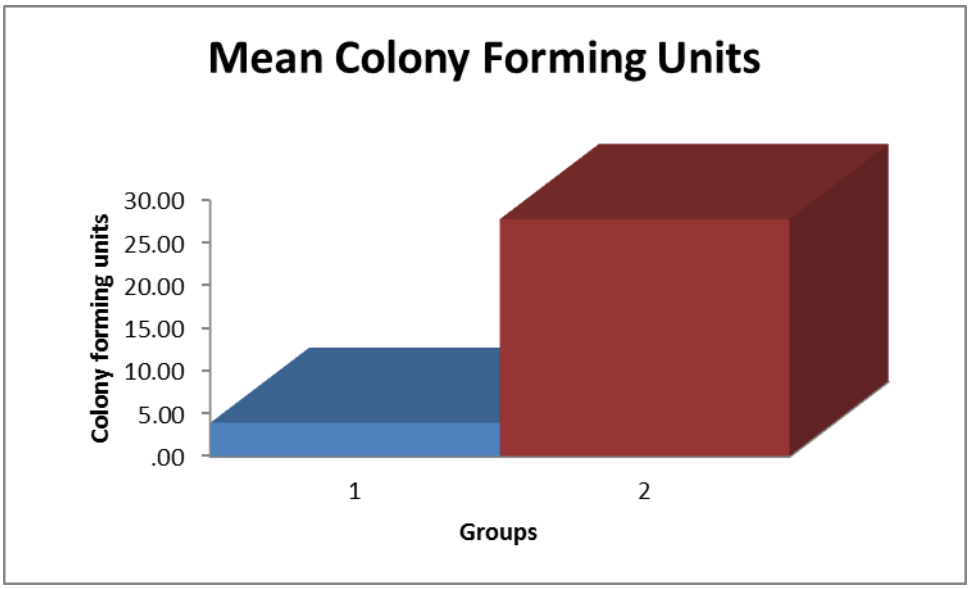
TABLE 4: Number of microbial colonies after Disinfection by Immersion technique in Group I B

Number of colonies seen after 72 hours at 10^{-4} dilution

S NO.	CFU/ml
1	20
2	35
3	28
4	32
5	24

The mean colony forming units counted after immersion of cast in disinfectant is 27.8 CFU/ml

GRAPH 1 . Comparative Bar Graph Showing Mean Colony Forming Units For Group I Microbial Study



GROUP II-COMPRESSIVE STRENGTH STUDY

The dry Compressive strength for the three groups of stone specimens after 24 hours was tested in universal testing machine at speed of 1mm per minute standardisation. The samples of three groups were transferred to Universal testing machine and specimens were stressed with increasing loads at the standard speed of 1mm per minute. The values obtained at fracture of each specimen were recorded.

TABLE 5.Breakage Forces of Group II A Test stone specimens after Disinfection by Incorporation technique.

SNO	$B_{F\ kg/cm2}$	$B_{f\ Mpa}$
1	183	18
2	176	17

3	234	23
4	267	26
5	124	12
6	183	18
7	285	28
8	229	22
9	275	27
10	256	26
11	295	29
12	244	24
13	265	26
14	285	28
15	224	22
16	183	18
17	142	14
18	234	23
19	254	25
20	152	15
21	295	29
22	182	18
23	254	25
24	214	21
25	224	22
26	295	29

27	132	13
28	275	27
29	285	28
30	183	18

The mean breakage force for GROUP II A is 22.mpa (224kg/cm^2)

TABLE.6. Breakage Forces Of Group II B Test stone specimens after Disinfection by Immersion technique.

SNO	$B_{F\ kg/cm2}$	$B_{f\ Mpa}$
1	193	19
2	194	19
3	216	21
4	164	16
5	166	16
6	214	21
7	185	18
8	204	20
9	193	19
10	173	17
11	234	23
12	193	19
13	183	18

14	244	24
15	244	24
16	193	19
17	183	18
18	214	21
19	203	20
20	214	21
21	214	21
22	193	19
23	142	14
24	183	18
25	173	17
26	224	22
27	152	15
28	193	19
29	254	25
30	214	21

The mean breakage force for GROUP II B is 19.mpa (198kg/cm^2)

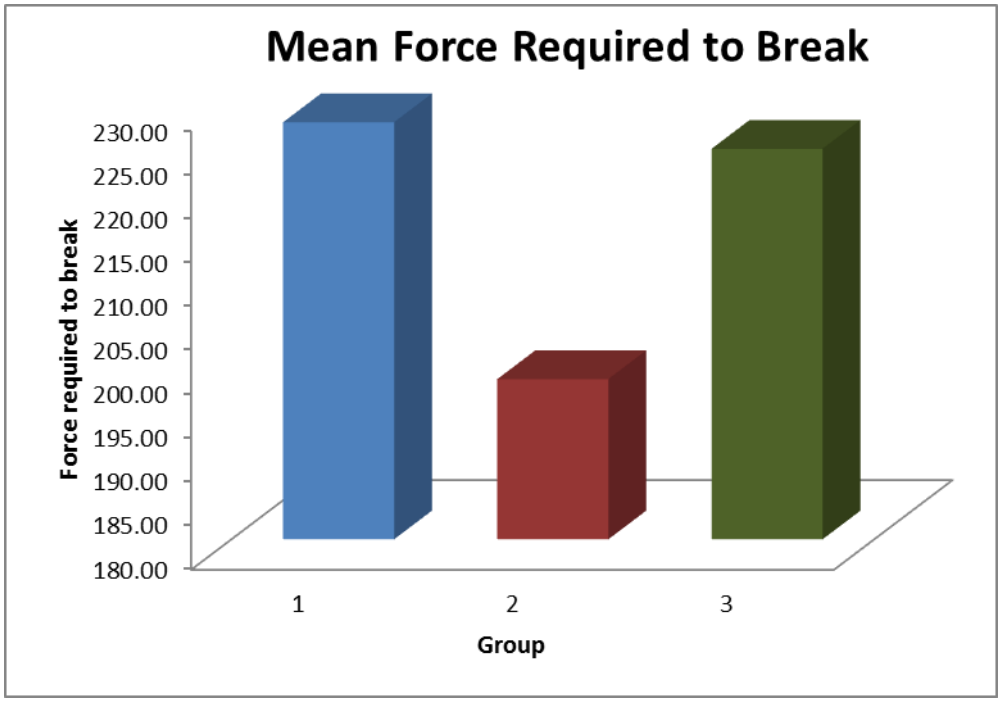
TABLE 7 .GROUP II C- Breakage Forces of Control Stone Specimens

SNO	$B_{F\ kg/cm2}$	$B_{f\ Mpa}$
1	285	28
2	278	27
3	204	20
4	256	25
5	267	26
6	195	19
7	236	23
8	224	22
9	193	19
10	204	20
11	224	22
12	163	16
13	193	19
14	224	22
15	224	22
16	234	23
17	203	20
18	285	28
19	265	26
20	244	24
21	275	27
22	254	25

23	265	26
24	214	21
25	193	19
26	183	18
27	122	12
28	132	13
29	275	27
30	224	22

The mean breakage force for GROUP II C is 22.37mpa (227.63kg/cm^2)

GRAPH . 2. Comparative Bar Graph Showing Mean Breakage Forces of Group II Compressive strength Study



Null Hypothesis

The two null hypotheses assumed were

- Null hypothesis $H_0$1: There is no difference between two disinfection methods (Immersion method and Incorporation method) on reduction in Microbial contamination on Dental stone casts models.

- Null hypothesis $H_0$2: There is no difference in the Compressive strength of dental stone models disinfected by Immersion technique and Incorporation technique.

STATISTICAL ANALYSIS

From the data obtained, the mean values and standard deviations were calculated. These results were subjected to statistical analysis to test the study hypothesis. Data was entered in spreadsheet and SPSS version 19 (IBM) was used (statistical package for social sciences) for data analysis. The normality of the data was checked using Kolmogorov Smirnov test.

MICROBIAL STUDY -DESCRIPTIVE DATA

Table.8. Descriptives for Microbiological Parameter (No. of Colony Forming Units [CFU]/ml)

Group I	Mean	Std. Deviation	Minimum	Maximum
I A	4	4.06	0	9
I B	27.8	6.02	20	35

The mean value of Group I A is 4. The mean value for group I B is 27.8. The mean CFU is higher for group B than group A. The standard deviation of group A is higher than the mean which signifies that there is a wide difference in the samples.

For Group I Microbial Study, Mann Whitney U Test was used for analysis as the sample size was less and did not follow normal distribution.

Table 9 . Comparison between The Groups For CFU/Ml

Group I	Mean ±SD	Mean difference	z test	p value
A	4 ± 4.06	-30.9655172413793	- 2.619	**0.009**
B	27.8 ± 6.02			

SD - Standard Deviation. P value < 0.05 is significant

z test and p value obtained from Mann Whitney U Test.

On comparing both the groups, it was found that the mean difference was statistically significant with p value < 0.

From the test of significance, it was clear that there is statistically significantly difference between group IA and group IB which rejects null hypothesis H0 1.

COMPRESSIVE STRENGTH STUDY

Table 10: Descriptives for required Breakage Forces (F Kg/ cm2)(N = 30)

Group II	Mean Force	Std Deviation	Lower Bound	Upper Bound	Minimum	Maximum
IIC	227.63	52.304	208.10	247.16	124	295
II B	198.30	26.713	188.33	208.27	142	254
II A	224.60	42.614	208.69	240.51	122	285

The mean Breakage force required was found to be 227.63+/-52.302 SD for Group II C. The mean Breakage force required was found to be 198.30 +/-26.713 SD for group II B. The mean force required to break was found to be mean 224.60+/-42.614 SD for group II A.

In Group II Compressive strength study, three subgroups were present; therefore One Way ANOVA was used for analysis

Table.11. Comparison between the groups for Breakage Forces required (kg/cm^2)

Group II	Mean ±SD	F value	p value
C	227.63 ± 52.30	4.448	**0.014**
B	198.30 ± 26.71		
A	224.60 ± 42.61		

SD - Standard Deviation. P value < 0.05 is significant
F value and p value obtained from One way ANOVA

In one way ANOVA, Since P value was less than 0.05, there was significant difference between the three groups. To find out which group contributes to statistically significant results Post Hoc test was applied.

Tukey's Honesty significant difference (HSD) is one among the post –Hoc test methods to do multiple pairwise comparisons. If the difference between groups mean is considerably bigger than the general variation, then it is inferred that there is a significant difference

Table 12. Post hoc analysis by Tukey's HSD – Multiple comparisons

Group II	Group II	Mean difference	p value
C	B	29.333*	**.022**
	A	3.033	.958
B	A	-26.300*	**.045**

P value < 0.05 is significant

In Post –hoc Analysis there was statistically significant difference between group C and Group B at P value of 0.022 and statistically significant difference between group B and group A at P value of 0.045. There was no statistically significant value between group A and group C.

From the test of significance, it was clear that there is statistically significant difference between group II C and group II B which rejects null hypothesis H0 2.

Hence both the null hypotheses were rejected.

Edentulousness is not a disease entity by itself, but rather a consequence of pathology. Increasing incidence of edentulousness over the recent years has questioned the adequacy of dental treatment. The treatment of these individuals with artificial prosthesis not only rehabilitates them functionally, but also in esthetically and psychologically. The mainstay for the management of completely edentulous or partially edentulous state, till date remains to be acrylic dentures.

It is well-known that the making of any prosthesis has multiple clinical and laboratory procedures .The cast models gets contaminated with microorganisms at various stages of prosthesis fabrication[8] like during jaw relation, trial, metal-try in and during post insertion check-up stages. The main source of contamination of dental casts in the dental laboratory is the contaminated denture base, metal copings and the master casts. This contaminated prosthesis can spread microorganisms to other materials, equipment, and personnel through contact or air during adjustments. Possible mechanisms of prevention of such spread have also been investigated.

According to the centre for Disease control[14], blood and saliva should be thoroughly and carefully cleaned from material that has been used in the mouth. Contaminated materials, impressions, stone models and intra-oral devices should also be cleaned and disinfected before being handled in the dental laboratory and before they are placed in the patient's mouth.

Sterilization is defined as the destruction or removal of all forms of life, with particular reference to microorganisms. The practical criterion of sterility is the absence of microbial growth in suitable media. Other criteria are loss of motility and inhibition of metabolism and particular enzymes. The ultimate requirement of sterilization is the destruction of bacterial and fungal spores. Agents capable of sterilizing are 1) steam

under high pressure, 2) high temperature including open flame, 3) filters, 4) ethylene oxide, 5) radiation and 6) certain chemicals.

Disinfection properly refers only to the inhibition or destruction of pathogens. By custom, the term disinfection is reserved for agents applied to inanimate objects. Bacteriostatic agents act by inhibiting the growth of microorganisms without killing them, as their effects are reversible. Conversely, bactericidal agents kill microorganisms by an action that is not reversible.

In our country disinfection of dental casts are not done as a routine procedure in the dental office or in the lab during the hustle of fabrication of prosthesis. It is therefore highly recommended that disinfection should be done at a standard time to keep denture bases and prosthesis free from microorganisms. From the patient aspect, routine standard disinfection procedure plays significant role in maintenance of good oral health. From clinical aspect it creates a healthy environment for medical practitioners and assistants.

Various studies indicate that the barrier systems are effective in reducing microbial contamination on dental casts. The chemical disinfection methods are commonly used to prevent cross contamination. Every dentist should be able to decide the choice of chemical disinfectant solution. It is important to have some concept of how micro-organisms are inhibited or killed, since the knowledge about the mode of action of particular agent makes it possible to decide how best to apply it. The antimicrobial agents act by obstructing critical metabolic functions by inhibiting or destroying essential microbial components. The antimicrobial mechanisms include protein denaturation, damage to cell membranes, inhibition of enzymes, alteration of nucleic material.

The BDA[6] recommends 70% isopropyl alcohol, hypochlorite solution (containing 1% available chlorine), or 2% glutaraldehyde solution for disinfecting contaminated surfaces under clinical contaminated circumstances. The disinfectant used in this study, is 2% glutaraldehyde. According to Rudd[26] et al (1986), glutaraldehyde has been shown to be effective against microorganisms including spores. Moreover it was effective against hepatitis virus. Only glutaraldehyde and povidone-iodine killed all contaminating microorganisms within one hour, while the 1:5 dilution of sodium hypochlorite solution was equally effective after twenty-four hours. 2% percent Glutaraldehyde was the most effective disinfectant with the least adverse effects on the physical properties of the set cast in a study done by Gibbs[12] (1990). Although povidone-iodine caused a decrease in the compressive strength of the set cast, it can be considered to be a sound alternative.

Glutaraldehyde is a saturated dialdehyde aqueous solution that has gained wide acceptance as a high-level disinfectant and chemical sterilant. The biocidal activity of glutaraldehyde results from its alkylation of sulfhydryl, hydroxyl, carboxyl, and amino groups of microorganisms, which alters RNA, DNA, and protein synthesis.

In an invitro study by Kambl[20] et al(1993), impressions were contaminated with known samples of microorganisms. Later the impressions were disinfected by immersion and cultured using swab methodology. Then the samples were assessed for bacterial colony and changes for surface detail reproduction of impression. Disinfection was good with reduction with microbial colony, but the surface detail reproduction of the cast was deteriorated.

For gypsum products quick method of disinfection in labs is spray technique. Vikas[46] et al(1991) evaluated and compared the effect of 0.5% chlorhexidine gluconate,

1% sodium hypochlorite, and 2% glutaraldehyde spray atomization technique on the linear dimensional stability. Results showed there was no change in the strength of plaster, yet the anti –microbial effect was significantly less.

The commonest method of disinfection is immersion technique being convenient and economic in choice. Here the impressions and casts are immersed in suitable disinfectant for an adequate length of time. The immersion protocol has to be repeated for master casts after every clinical procedure.

Moslehifard, Elnaz[27] et al (2015) evaluated the changes in dimensional accuracy and hardness of the dental casts after repeated disinfection in 0.525% sodium hypochlorite and 2% glutaraldehyde solutions. Results demonstrated that repeated immersion of type III dental stone specimens in slurry with distilled water, 0.525% sodium hypochlorite and 2% glutaraldehyde, along with drying in air, caused a significant increase in linear dimension and a significant decrease in hardness.

Various literary reviews have evaluated the effectiveness of disinfecting solutions incorporated into elastomeric impression materials against a standard and representative group of microorganisms and to note changes in the physical properties of the casts. Towards the results, the surface deterioration was not so significant in casts, yet in-vivo patient tolerance to disinfectant in impression material was poor.

Review of literature describes numerable disinfection procedures with various disinfectants. Most of the studies focus only on disinfection of impressions. Only few states about immersion disinfection of gypsum casts. As per protocol, repeated immersion of master cast has to be followed after every clinical step. Incorporation technique is single time disinfection. There are no studies comparing incorporation of 2% glutaraldehyde with immersion technique in dental stone.

This thesis study was carried out to compare the efficacy of two disinfection methods and compare the properties of Gypsum type III Dental stone models disinfected by Immersion technique and Incorporation technique using 2% Glutaraldehyde. The reduction in microbiological contamination level and compressive strength of stone models were analysed in these two disinfection procedures.

The methodology of microbial study chosen was based on literary review of Gloria[13] et al (2014). Ten impressions were made on nissin 200-m typodont upper jaw with alginate. Impressions were intentionally contaminated with known microorganisms.

In a Study by Mitchell[25] et al (1997), it was found that not only oral Streptococci was present on the surface of impression, but also the Candida, MRSA, and P aeruginosa species were found. These microbes can be recovered readily from gypsum casts 24 hours following the pouring of the impression. So these organisms were evaluated in the present study.

Ten impressions were divided into two groups (IA ,IB) and casts were poured. These casts were subjected to Incorporation disinfection and Immersion disinfection[45] with 2% Glutaraldehyde respectively. Within each group, samples were scrapped from the cast and cultured to examine for colonization. The samples were then inoculated in the Human blood agar and Mc.conkey agar culture plate. All instruments and equipments were handled in aseptic manner during the test. The agar plates were incubated at 37 º C for 72 hours. The microbial colony count was read using a 4X magnification lens. The specimens were observed for the microbial colonies and some interesting results were obtained.

The Microbial colony count was found to be significantly different between group I A and group I B (Table 3, 4). The colony count was higher in the Immersion disinfection .Among the two types of disinfection method used in this study; it was observed that samples from Immersion disinfection methods showed more colony forming units.

The mean CFU was higher for Group I B (27) than Group I A (4). The standard deviation of group IA is higher than the mean which signifies that there is a wide variation in the sample (Table 8). On comparing Group IA and Group I B with Mann Whitney U test (Table 9), it was found that the mean difference was statistically significant with p value < 0.001.

The methodology for Compressive strength study chosen was based on a literature review by Hota et al (2014). In the present study 90 gypsum test specimens were made. They were divided into three groups – II A, II B, II C. The Group II A test specimens were subjected to Incorporation disinfection. The Group II B Test specimens were subjected to Immersion disinfection. The Group II C stone specimens were kept as control group without disinfection.

According to Van Noort R et al (1989), first hour after mixing, the compressive strength is the measure of wet strength, while gypsum may take as long as 7 days to dry. For practical purposes stone casts would reach sufficient hardness after 24 hours. There is no improvement in abrasion resistance between 24 hours and 7 days air.

In this study after 24 hours the stone specimens were tested for dry compressive strength. The test specimens of three groups were transferred to Universal

testing machine and specimens were stressed with increasing loads up till it fractures to evaluate their dry compressive strength

The mean breakage force for GROUP II A is 22mpa (Table 4). The mean breakage force for GROUP II B is 19mpa (Table 5). The mean breakage force for GROUP II C is 22.37mpa (Table 6). In Group II Compressive strength study, as three subgroups are present; One Way ANOVA was used for analysis. On comparing the three groups it was found that the mean difference was statistically significant with F-value of 4.448 and p = 0.014(Table 11). In one way ANOVA, Since P value is less than 0.05, there is significant difference between the three groups. To find out which group contributes to statistically significant results Post Hoc test was applied (Table 12). In Post –hoc Analysis there was statistically significant difference between group C and Group B at P value of 0.022 and statistically significant difference between group B and group A at P value of 0.045. There was no statistically significant value between group A and group C.

The recommended mean Compressive strength for type III Gypsum Dental stone is 20-35mpa The result of the current study showed that the process of Immersing Type III Dental stone cylindrical specimens(GROUP II B 19mpa) in disinfectant (2% Glutaraldehyde) and subsequent drying reduced the Compressive strength significantly when compared with the control specimens(GROUP II C 22.37mpa). There was interaction between Stone specimens (GP II A 22mpa)and Disinfectant solution in Incorporation disinfection which only minimally affected the dry Compressive strength of Dental stone.

Hence, in this study, it is proved that Incorporation of 2% Glutaraldehyde solution into Dental stone cast is an effective disinfection method to be followed in the dental clinics and the laboratories, which reduces the chances of cross contamination.

LIMITATIONS OF THE STUDY

1. This is an in-vitro study which cannot mimic the complete oral microbial environment.
2. The intentional contamination is limited only to three micro-organisms Candida albicans, Pseudomonas aeruginosa, Staphylococcus aureus
3. The thesis study is limited only to 2% Glutaraldehyde as disinfectant..
4. In this study only two disinfection methods- Immersion and Incorporation techniques are followed.
5. The study is limited to only one gypsum product type III Dental stone, which is commonly used for fabrication of master cast.

The results in a nutshell within the limitations of this study can be stated as follows.

Incorporation of 2% Glutaraldehyde during type III Gypsum product model preparation achieved higher level of disinfection with favourable dry Compressive strength when compared to 10 minutes of Immersion of type III Gypsum model. Therefore 2% Glutaraldehyde solution can be recommended in use for Incorporation disinfection of Dental stone.

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